Methods: We collected six age - and sex-matched probes of human cartilage before undergoing total knee joint replacement under from OA patients. Cells from macroscopic "normal" and arthritic areas were harvested from each knee. Tissue specimens were extracted under sterile conditions and one part immediately snap frozen in liquid nitrogen for methylation array (Human CpG Island Microarray Kit, Agilent Inc., California, USA). The other part was cultured. After digestion, the cells were treated with 10 uM of demethylation agent, 5-AZA-deoxy-cytidine, over a period of six days. DNA was isolated using Qiagen QIAamp DNA Micro Kit (QIAGEN Inc., California, USA) according to manufacturer's protocol. After harvesting the cells, RNA was extracted using the Trizol method and cDNA was transcribed. Gene expression was performed with the Taqman® Realtime PCR Assay using standardized primers.
Results: After analyzing microarray data 1246 genes, where of 936 have been identified, showed statistical differences between arthritic and non-arthritic areas. According to the p-value ($p<0,5$) and geometric value (arthritic/non arthritic) we picked out the following candidate genes; *GLA*, *ADORA3*, *LAMA4*, *ORMDL2*, *PRMT5*, *WDR46*. After treatment with 5-Aza-deoxy-cytidine we observed a significant increase of the candidate genes expression in human arthritic cartilage cell cultures compared to the untreated macroscopically intact controls.
Conclusions: The considerable increase of the investigated gene's expression in cell culture that correlated with the data gained from the methylation specific array lead us to the thought, that methylation may play an important role in the pathogenesis of OA. Further research in the field of microarray technique, including protein data, may lead us to therapeutic aspects of OA in the future.

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ECM REMODELLING AFFECTS SOX9-GSK3beta DEPENDENT CONTROL OF beta-CATENIN IN CHONDROCYTES

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Purpose: Our recent findings indicate that MMP-13 or IKKalpha KD impaired ECM remodeling resulted in pronounced alterations in the pattern of Sox9 nuclear localization along with changes in beta-catenin levels and activation status and disruption of chondrocyte differentiation. To mechanistically investigate crosstalk between ECM remodeling and beta-catenin, we evaluated the expression of GSK3beta (a key component of the beta-catenin degradation complex) in MMP-13 and IKKalpha KD micromasses and in cartilage samples with different degrees of ECM remodeling and the effects of inhibiting GSK3beta activity on chondrocyte differentiation.
Methods: The subcellular distribution and co-localization of Sox9 and GSK3beta were investigated in 1 week micromasses by confocal microscopy on sections passing through nuclei. In addition, GSK3beta expression was assessed in immunoblots of 1 week micromass lysates, upon normalization against GAPDH and its Sox9 interaction was also investigated in co-immunprecipitation experiments in conjunction with western blotting. Full thickness slices of cartilage from either normal/early OA or late OA cartilage samples were also analyzed for GSK3beta expression in conjunction with ECM status by Safranin-O staining. The phenotypic effects of inhibiting GSK3beta were assessed by comparisons of control and 5mM LiCl - treated high density monolayer cultures (in 5-replicates) and micromasses maturing over a 1-3 week time course.
Results: GSK3beta staining was predominantly nuclear in control, MMP-13 KD and IKKalpha KD micromass samples. However, unlike the GL2 controls, nuclear Sox9 co-localized with GSK3beta in MMP-13 KD and IKKalpha KD chondrocytes. Surprisingly, western blotting revealed higher levels of GSK3beta expression in IKKalpha KD micromasses, part of which immunoprecipitations showed to be in complexes with Sox9. Noteworthy, in cartilage tissue with strong safranin-O staining, GSK3beta expression was also markedly higher in middle zone chondrocytes. Chondrocytes cultured in the presence of 5 mM LiCl deposited a higher amounts of calcium as judged by spectrophotometric analysis of alizarin red staining. Higher levels of MMP-13 activity (as indicated by stronger expression of its processed, active form in western blots) were also observed in maturing micromasses with impaired GSK3beta activity. The greater extent of ECM remodeling in LiCl treated micromasses was in keeping with their reduced size and accelerated terminal differentiation.
Conclusions: We find that the impaired ECM remodeling in MMP-13 or IKKalpha KD in micromasses is associated with reduced levels of active beta-catenin and the presence of enhanced levels of nuclear Sox9 in complexes with GSK3beta. Moreover, middle zone cartilage with more intact ECM also presents a greater degree of nuclear Sox9 in association with higher levels of GSK3beta. Taken together these data suggest that prior to their undergoing ECM remodeling and subsequent terminal differentiation chondrocytes have a significant amount of their GSK3beta in nuclear complexes with Sox9, which could contribute to enhanced beta-catenin degradation as also recently reported in other cellular models. Sox9 dependent nuclear localization of the beta-catenin degradation complex may be an important component of the molecular constraints that maintain articular chondrocytes in their proper arrested state in healthy cartilage.
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HIGH MOLECULAR WEIGHT HYALURONIC ACID INHIBITS IL-6-INDUCED MATRIX METALLOPROTEINASES PRODUCTION BY UP-REGULATING THE EXTRACELLULAR SIGNAL-REGULATED KINASE INHIBITOR, MITOGEN-ACTIVATED PROTEIN KINASE PHOSPHATASE-1

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Purpose: To investigate the mechanism of the inhibitory action of high molecular weight hyaluronic acid (HA) on production of matrix metallopoteinases (MMPs) induced by IL-6 in human articular chondrocytes.
Methods: Human articular chondrocytes were stimulated by IL-6 and soluble IL-6 receptor (sIL-6R) with or without HA for 24 h and the production of MMP-1, MMP-3 and MMP-13 was measured. Phosphorylation of extracellular signal-regulated kinase (ERK), signal transducer and activator of transcription (STAT) and mitogen-activated protein kinase kinase (MEK) in IL-6+sIL-6R-treated chondrocytes was detected by Western blotting. Expression levels of mitogen-activated protein kinase phosphatase (MKP-1) in HA-treated chondrocytes were assessed by real-time PCR and Western blotting.
Results: IL-6 alone did not induce MMP-1, MMP-3 and MMP-13 production in human articular chondrocytes. However, in combination with sIL-6R, IL-6 increased MMP production in a concentration-dependent manner. Anti-IL-6R antibody suppressed MMP production induced by IL-6+sIL-6R. We examined the effects of parthenolide (PAR, a STAT inhibitor) and PD98059 (PD, a MEK-ERK inhibitor) on the production of MMP-1, MMP-3 and MMP-13 induced by IL-6+sIL-6R. After pre-incubation with PAR or PD, chondrocytes were stimulated with IL-6+sIL-6R for 24 h and then MMP-1, MMP-3 and MMP-13 concentrations were measured. IL-6+sIL-6R significantly induced MMP-1, MMP-3 and MMP-13 productions and suppression of the MEK-ERK signal pathway by PD resulted in a marked decrease of IL-6+sIL-6R-induced MMP-1, MMP-3 and MMP-13 production. In contrast, PAR only slightly decreased the production of MMPs from chondrocytes. Previously we found that HA inhibited IL-6-induced MMP production in chondrocytes. We examined if anti-CD44 antibody or anti-ICAM1 antibody reversed HA-induced suppression of MMP production by IL-6+sIL-6R. IL-6+sIL-6R showed remarked increase of MMP production and HA significantly suppressed IL-6+sIL-6R-induced MMP production. The inhibitory effect of HA on IL-6+sIL-6R-induced MMP production was decreased by anti-CD44 antibody, but not anti-ICAM-1 antibody. To further analyze the inhibitory action of HA on MAPK signal pathways, we tested whether HA blocked the phosphorylation of MEK and ERK. IL-6+sIL-6R promoted the phosphorylation of both MEK and ERK. However, HA treatment reduced the phosphorylation of ERK, but not MEK. Then we examined if HA could induce MKP-1, a negative regulator of ERK1/2. HA treatment induced the expression of MKP-1 mRNA in untreated chondrocytes. We also confirmed by Western blotting that MKP-1 protein was induced by HA in IL-6+sIL-6R-treated or untreated chondrocytes.
To determine the involvement of MKP-1 in HA-induced suppression of MMP production, we tested whether MKP-1 inhibitor blocked the effect of HA. MKP-1 inhibitor clearly reversed the suppressive effect of HA on MMP production, to the level of the negative control.
Conclusions: Our study clearly demonstrated that HA suppressed IL-6-induced MMP production by chondrocytes via MKP-1 induction through CD44 signaling.